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(54) Title: METHODS OF TREATING COGNITIVE DECLINE DISEASE CONDITIONS WITH ANDROGENS

(57) Abstract: Methods for treating a host, particularly a female host, for a cognitive decline disease condition are provided. Also provided are methods for improving the cognitive function of a host. In the subject methods, an effective amount of androgenic agent, e.g., a male sex hormone such as testosterone or analogue thereof, is administered to the host, resulting in at least an improvement in cognitive function of the host. The subject methods find use in a variety of different applications, and are particularly suited for use in treating female hosts for neurodegenerative disease conditions, e.g., Alzheimer's disease. Also provided are kits for use in practicing the subject methods.

METHODS OF TREATING COGNITIVE DECLINE DISEASE

CONDITIONS WITH ANDROGENS

INTRODUCTION

5 Field of the Invention

The field of this invention is cognition and methods for enhancing the same, e.g., for treatment of cognitive decline disease conditions, such as neurodegenerative disease conditions.

10 Background

Alzheimer's Disease (AD) is a progressive, neurodegenerative disease characterized by cognitive and non-cognitive behavioral changes which include: (a) memory loss; (b) language deterioration; (c) impaired visuo-spatial skills; (d) poor judgment; (e) indifferent attitude and (f) aimless, unpredictable behavior. Although AD usually begins after age 60, its onset may occur decades earlier. AD first appears as memory decline. As the disease progresses over several years, cognition, personality, and the ability to function are all impaired or destroyed. Confusion and restlessness may also occur. The type, severity, sequence, and progression of mental changes vary widely among AD patients. Some people have the disease for only the last 5 years of life, while others may have it for as many as 20 years.

There is no known cure for AD and no way to slow the progression of the disease. For some people in the early or middle stages of the disease, medication such as tacrine may alleviate some cognitive symptoms. Also, some medications may help control non-cognitive behavioral symptoms such as sleeplessness, agitation, wandering, anxiety, and depression. These treatments are aimed solely at making the patient more comfortable and do nothing to slow the progression of the underlying disease.

Increasingly, studies are reporting that estrogen may protect against Alzheimer's disease. A number of studies have reported that women taking hormone replacement therapy (in various combinations) score better on memory and learning than women not on HRT and have slower decline in mental functioning. Clinical trials are being conducted in patients with AD to evaluate the benefit of estrogen in females and of testosterone in males [Kampen, 1994 #7890; Cummings, 1998 #9342]. While estrogen treatment seems unable to slow disease progression (propagation phase), some studies suggest that it may delay or prevent the onset

of AD (initiation phase) [Yaffe, 2000 #12056]. Two studies found that women who took HRT had a reduced risk for Alzheimer's disease -- in one the risk was lower by 60%. These reports are backed up by studies indicating that estrogen stimulates blood flow in the brain, increases production of a beneficial form of beta amyloid, and triggers the temporary growth 5 of nerve pathways in the memory portion of the brain. However, estrogen had no beneficial effect on cognitive function in healthy elderly women that carry the *APOE ε4* allele.

As is evident from the above discussion, there is a continued need for the development of new treatment modalities for individuals suffering from Alzheimer's disease and other neurodegenerative disorders.

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Relevant Literature

U.S. Patents of interest include: 5,508,167; 5,554,601; 5,716,828; 5,767,248; 5,776,923; 5,780,587; 5,795,883; 5,840,540; 5,889,042; 5,986,054; 5,935,781; 6,027,896; and 6,027,899. Also of interest are U.S. Patent No. 6,175,057 and Raber et al., Proc. Nat'l 15 Acad. Sci. USA (1998) 95: 10914-10919.

SUMMARY OF THE INVENTION

Methods for treating a host, particularly a female host, for a cognitive decline disease condition are provided. Also provided are methods for improving the cognitive function of a 20 host. In the subject methods, an effective amount of an androgenic agent, e.g., a male sex hormone such as testosterone or analogue thereof, is administered to the host, resulting in at least an improvement in cognitive function of the host. The subject methods find use in a variety of different applications, and are particularly suited for use in treating female hosts for neurodegenerative disease conditions, e.g., Alzheimer's disease. Also provided are kits 25 for use in practicing the subject methods.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 provides a graphical depiction of the results observed using the water maze assay described in the Experimental section, infra.

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DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods for treating a host, particularly a female host, for a cognitive decline disease condition are provided. Also provided are methods for improving the cognitive function of a

host. In the subject methods, an effective amount of androgenic agent, e.g., a male sex hormone such as testosterone or analogue thereof, is administered to the host, resulting in at least an improvement in cognitive function of the host. The subject methods find use in a variety of different applications, and are particularly suited for use in treating female hosts 5 for neurodegenerative disease conditions, e.g., Alzheimer's disease. Also provided are kits for use in practicing the subject methods.

Before the subject invention is further described, it is to be understood that the invention is not limited to the particular embodiments of the invention described below, as 10 variations of the particular embodiments may be made and still fall within the scope of the appended claims. It is also to be understood that the terminology employed is for the purpose of describing particular embodiments, and is not intended to be limiting. Instead, the scope of the present invention will be established by the appended claims.

15 In this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs.

20 METHODS

In the broadest sense, methods are provided for improving a cognitive function in a mammalian host. In practicing the subject methods, an effective amount of an androgenic agent is administered to the host to obtain the desired improvement in cognitive function. The host is generally a mammalian host, and in many embodiments is a human. In certain 25 preferred embodiments, the host is a female host, e.g., a female human. In certain preferred embodiments, the host comprises at least one ApoE4 allele, such that it expresses the ApoE4 isoform and includes the ApoE4 isoform. As such, the host may be heterozygous or homozygous for the ApoE4 isoform. In certain preferred embodiments, the host is homozygous for ApoE4. In these embodiments, the host may be a male host that carries the 30 ApoE4 allele, but in many embodiments is a female host.

In practicing the subject methods, an effective amount of androgenic agent is administered to the host. Suitable androgenic agents that may be used in the subject methods include, but are not limited to: the naturally occurring androgens and derivatives thereof

including androsterone, androsterone acetate, androsterone propionate, androsterone benzoate, androstenediol, androstenediol-3-acetate, androstenediol-17-acetate, androstenediol-3,17-diacetate, androstenediol-17-benzoate, androstenediol-3-acetate-17-benzoate, androstenedione, dehydroepiandrosterone (DHEA; also termed "prasterone"),

5 sodium dehydroepiandrosterone sulfate, 4-dihydrotestosterone (DHT; also termed "stanolone"), 5 α -dihydrotestosterone, dromostanolone, dromostanolone propionate, ethylestrenol, nandrolone phenpropionate, nandrolone decanoate, nandrolone furylpropionate, nandrolone cyclohexanepropionate, nandrolone benzoate, nandrolone cyclohexanecarboxylate, oxandrolone, stanozolol and testosterone; pharmaceutically acceptable esters of testosterone and 4-dihydrotestosterone, typically esters formed from the hydroxyl group present at the C-17 position, including, but not limited to, the enanthate, propionate, cypionate, phenylacetate, acetate, isobutyrate, buciclate, heptanoate, decanoate, undecanoate, caprate and isocaprate esters; and pharmaceutically acceptable derivatives of testosterone such as methyl testosterone, testolactone, oxymetholone and fluoxymesterone.

10 The aforementioned testosterone esters are commercially available or may be readily prepared using techniques known to those skilled in the art. (Generally, the 17-hydroxyl group of the testosterone molecule is caused to react with a suitable organic acid under esterifying conditions, such conditions typically involving the use of a strong acid such as sulfuric acid, hydrochloric acid, or the like, and a temperature sufficient to allow the reaction

15 to proceed at reflux.)

The androgenic agent is generally administered to the host as a pharmaceutical composition that includes an effective amount of the androgenic agent in a pharmaceutically acceptable vehicle. In the subject methods, the active agent(s) may be administered to the host using any convenient means capable of resulting in the desired improvement on cognitive function. Thus, the agent can be incorporated into a variety of formulations for therapeutic administration. More particularly, the agents of the present invention can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, topical patches and aerosols.

As such, administration of the agents can be achieved in various ways, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, transdermal, intracheal, topical, etc., administration. In pharmaceutical dosage forms, the agents may be administered

in the crystalline form or in the form of their pharmaceutically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

5 For oral preparations, the agents can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with 10 lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

The agents can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or 15 propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

The agents can be utilized in aerosol formulation to be administered via inhalation. The compounds of the present invention can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.

20 Furthermore, the agents can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. The compounds of the present invention can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

25 Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more inhibitors. Similarly, unit dosage forms for injection or intravenous administration may comprise the inhibitor(s) in a composition as a solution in sterile water, 30 normal saline or another pharmaceutically acceptable carrier.

Topical preparations are also of use. Topical formulations of androgenic compositions are known in the art. See e.g., U.S. Patent Nos. 5,622,944; 5,635,203; 5,785,991 and 5,882,676; the disclosures of which are herein incorporated by reference.

The term "unit dosage," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present invention depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

Those of skill will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

Administration of the androgenic agent to the host according to the subject methods results in an improvement in at least one cognitive function of the host. Cognitive functions of interest include: spatial and non-spatial learning and memory, and the like. In certain situations, treatment according to the subject methods results in a complete removal of a deficit in the cognitive function. The amount of improvement is at least about 2 fold, usually at least about 5 fold and more usually at least about 10 fold as compared to a suitable control, e.g., an otherwise substantially identical host not administered an androgenic agent, e.g., a female host having similar level of cognitive ability (e.g., suffering from the same cognitive decline disease condition such as Alzheimer's disease, etc.) that has been administered a placebo, where in certain embodiments the amount of improvement is at least about 25 fold, 50 fold, 75 fold, 100 fold or greater. The cognitive function improvement can be evaluated using any convenient protocol, where suitable protocols include, but are not limited to: 1. Wechsler Adult Intelligence Scale(WAIS_-R) [Wechsler, D. WAIS-R Manual. New York: Psychological Corporation, 1981; Ivnik et al.. Mayo's older american normative studies:WAIS-R norms for age 56 to 97. Clin Neuropsychol 1992, 6 (suppl):1-30]; 2. Mini-Mental State Examination (MMSE) [Folstein et al. Mini Mental State: a practical method for grading the cognitive state of patients for the clinician. J Psychiat Res 1975;12:189-98; Commenges et al. Statistical description of the Mini-Mental State Examination (MMS) for

French elderly community residents. *J Nerv Ment Diss* 1992;180:28-32, 3. Information-Memory-Concentration test [Blessed et al. The association between quantitative measures of dementia and of senile changes in the cerebral grey matter of elderly subjects. *Br J Psychiat* 1968;114:796-811], 3. Fuld Object Memory Evaluation (FOSE) [Fuld, PA. The Fuld Object Memory Test. Chicago: The Stoelting Instrument Company, 1981], 4. The Buschke Selective Reminding Test (BSRT) [Buschke, H. Selective reminding for analysis of memory and learning. *J Verbal Learn Verb Behav* 1973;12:543-50], 5. The Rey Auditory Recall Test [Buschke, H. Selective reminding for analysis of memory and learning. *J Verbal Learn Verb Behav* 1973;12:543-50], 6. The CERAD Word List [Morris et al. The consortium to 5 establish a registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's Disease. *Neurology* 1989;39:1159-65], 7. The Beton Visual Retention Test (BVRT) [Benton, AL. The revised visual attention test, 4th edn. New York: Psychological Corporation, 1974], 8. The California Verbal Learning Test [Delis et al. The California Verbal Learning Test. New York: Psychological Corporation, 10 15 1987], 9. Assessment of navigation in humans [Maguire et al. Knowing where and getting there; a human navigation network [Science 1998; 280:921-924]

In certain embodiments, the subject methods further include a step of evaluating whether the host carries an allele for the human ApoE4 isoform. A variety of different methods are known and currently available to those of skill in the art for detecting the 20 presence of the human ApoE4 allele in a host. Representative methods are now described in greater detail.

The step of detecting the presence or absence of ApoE4 or of DNA encoding such isoform (including the number of alleles for ApoE4) may be carried out either directly or indirectly by any suitable means. A variety of techniques are known to those skilled in the 25 art. All generally involve the step of collecting a sample of biological material containing either DNA or ApoE from the subject, and then detecting whether or not the subject possesses ApoE4 or DNA encoding such isoform from that sample. For example, the detecting step may be carried out by collecting an ApoE sample from the subject (for example, from cerebrospinal fluid, plasma, or any other fluid or tissue containing ApoE), 30 and then determining the presence or absence of an ApoE4 isoform in the ApoE sample (e.g., by-isoelectric focusing, western blot or dot blot analysis, or immunoassay).

The isolation and characterization of ApoE is described, for example, in Rall et al., *Methods in Enzymology* 128:273-287 (1986), Davignon et al., *Arteriosclerosis* 8:1-21

(1988), and in Warnick et al., Clin. Chem. 25:279-284 (1979), all of which are incorporated by reference herein. Isoelectric focusing is an electrophoretic technique by which the molecules are separated based on their isoelectric points (pI) along a continuous pH gradient. Reference proteins, commercially available (e.g., Sigma Chemical Company, St. Louis, Mo.), are used to indicate a gradient along which the sample proteins match up according to where their pH matches their pI. In Warnick, et al. very-low-density apolipoproteins are isolated from plasma samples and applied to isoelectric focusing gels and the isoelectric focusing patterns of the ApoE isoforms are obtained. According to the Warnick et al. procedure, pI values of the ApoE isoform E4 was about 6.1 respectively in 8M urea at 4° C. See also, Pagnan et al., J. Lipid. Res. 18:613- 622 (1977) and Utermann et al., FEBS Lett. 56:352-355 (1975). Various isoelectric focusing-type techniques are also provided in Rall et al. *supra*, including analytical isoelectric focusing, cysteamine treatment, neuraminidase treatment, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, as well as amino acid analysis and nucleic acid sequence analysis, and capillary isoelectric focusing is described in H. Swartz, Bio/Technology 12, 408-09 (April 1994).

In the alternative, the detecting step may be carried out by collecting a biological sample containing DNA from the subject, and then determining the presence or absence of DNA encoding an ApoE4 isoform in the biological sample. Any biological sample which contains the DNA of that subject may be employed, including tissue samples and blood samples, with blood cells being a particularly convenient source. The amino acid sequences and nucleic acid sequences ApoE4 are known and described. See, for example, Paik et al., Proc. Nat'l Acad. Sci. U.S.A. 82:3445-3449 (1985) for the nucleic acid sequence Mahley, Science, 240:622-630 (1988) for the amino acid sequence information.

Determining the presence or absence of DNA encoding an ApoE4 isoform may be carried out with an oligonucleotide probe labeled with a suitable detectable group, or by means of an amplification reaction such as a polymerase chain reaction or ligase chain reaction (the product of which amplification reaction may then be detected with a labeled oligonucleotide probe or a number of other techniques). Further, the detecting step may include the step of detecting whether the subject is heterozygous or homozygous for the gene encoding an ApoE4 isoform. Numerous different oligonucleotide probe assay formats are known which may be employed to carry out the present invention. See, e.g., U.S. Pat. No. 4,302,204 to Wahl et al.; U.S. Pat. No. 4,358,535 to Falkow et al.; U.S. Pat. No. 4,563,419 to

Ranki et al.; and U.S. Pat. No. 4,994,373 to Stavrianopoulos et al. (applicants specifically intend that the disclosures of all U.S. Patent references cited herein be incorporated herein by reference).

Amplification of a selected, or target, nucleic acid sequence may be carried out by 5 any suitable means. See generally D. Kwong and T. Kwong, Am. Biotechnol. Lab. 8, 14-25 (1990). Examples of suitable amplification techniques include, but are not limited to, polymerase chain reaction, ligase chain reaction, strand displacement amplification (see generally G. Walker et al., Proc. Natl. Acad. Sci. U.S.A. 89, 392-396 (1992); G. Walker et al., Nucleic Acids Res. 20, 1691-1696 (1992)), transcription-based amplification (see D. 10 Kwong et al., Proc. Natl. Acad. Sci. U.S.A. 86, 1173-1177 (1989)), self-sustained sequence replication (or "3SR") (see J. Guatelli et al., Proc. Natl. Acad. Sci. U.S.A. 87, 1874-1878 (1990)), the Q β replicase system (see P. Lizardi et al., BioTechnology 6, 1197-1202 (1988)), nucleic acid sequence-based amplification (or "NASBA") (see R. Lewis, Genetic 15 Engineering News 12 (9), 1 (1992)), the repair chain reaction (or "RCR") (see R. Lewis, supra), and boomerang DNA amplification (or "BDA") (see R. Lewis, supra). Polymerase chain reaction is currently preferred. DNA amplification techniques such as the foregoing can involve the use of a probe, a pair of probes, or two pairs of probes which specifically bind to DNA encoding ApoE4, but do not bind to DNA encoding ApoE2 or ApoE3 under the same hybridization conditions, and which serve as the primer or primers for the 20 amplification of the ApoE4 DNA or a portion thereof in the amplification.

In general, an oligonucleotide probe which is used to detect DNA encoding ApoE4 is an oligonucleotide probe which binds to DNA encoding ApoE4, but does not bind to DNA encoding ApoE2 or ApoE3 under the same hybridization conditions. The oligonucleotide probe is labeled with a suitable detectable group, such as those set forth below in connection 25 with antibodies.

Polymerase chain reaction (PCR) may be carried out in accordance with known techniques. See, e.g., U.S. Pat. Nos. 4,683,195; 4,683,202; 4,800,159; and 4,965,188. In general, PCR involves, first, treating a nucleic acid sample (e.g., in the presence of a heat stable DNA polymerase) with one oligonucleotide primer for each strand of the specific 30 sequence to be detected under hybridizing conditions so that an extension product of each primer is synthesized which is complementary to each nucleic acid strand, with the primers sufficiently complementary to each strand of the specific sequence to hybridize therewith so that the extension product synthesized from each primer, when it is separated from its

complement, can serve as a template for synthesis of the extension product of the other primer, and then treating the sample under denaturing conditions to separate the primer extension products from their templates if the sequence or sequences to be detected are present. These steps are cyclically repeated until the desired degree of amplification is

5 obtained. Detection of the amplified sequence may be carried out by adding to the reaction product an oligonucleotide probe capable of hybridizing to the reaction product (e.g., an oligonucleotide probe of the present invention), the probe carrying a detectable label, and then detecting the label in accordance with known techniques, or by direct visualization on a gel. When PCR conditions allow for amplification of all ApoE allelic types, the types can be

10 distinguished by hybridization with allelic specific probe, by restriction endonuclease digestion, by electrophoresis on denaturing gradient gels, or other techniques. A PCR protocol for determining the ApoE genotype is described in Wenham et al., *The Lancet* 337:1158-1159 (1991), incorporated by reference herein. Examples of primers effective for amplification and identification of the ApoE isoforms are described therein. Primers specific

15 for the ApoE polymorphic region (whether ApoE4, E3 or E2) can be employed. In Wenham, for example, PCR primers are employed which amplify a 227 bp region of DNA that spans the ApoE polymorphic sites (codons 112 and 158, which contain nucleotides 3745 and 3883). The amplified fragments are then subjected to restriction endonuclease CfoI which provides different restriction fragments from the six possible ApoE genotypes which may be

20 recognizable on an electrophoresis gel. See also, Hixon et al., *J. Lipid Res.* 31:545-48 (1990); Houlston et al., *Hum. Genet.* 83:364-365 (1989) Wenham et al., *Clin. Chem.* 37:241-244 (1991); and Konrula et al., 36:2087-92 (1990) for additional methods, all of which are incorporated by reference herein.

Ligase chain reaction (LCR) is also carried out in accordance with known techniques.

25 See, e.g., R. Weiss, *Science* 254, 1292 (1991). In general, the reaction is carried out with two pairs of oligonucleotide probes: one pair binds to one strand of the sequence to be detected; the other pair binds to the other strand of the sequence to be detected. Each pair together completely encompasses the strand to which it corresponds. The reaction is carried out by, first, denaturing (e.g., separating) the strands of the sequence to be detected, then reacting

30 the strands with the two pairs of oligonucleotide probes in the presence of a heat stable ligase so that each pair of oligonucleotide probes is ligated together, then separating the reaction product, and then cyclically repeating the process until the sequence has been

amplified to the desired degree. Detection may then be carried out in like manner as described above with respect to PCR.

As an alternative to isoelectric focusing and techniques for allele detection, the step of determining the presence or absence of the ApoE4 isoform in a sample may be carried out 5 by an antibody assay with an antibody which selectively binds to ApoE4 (i.e., an antibody which binds to ApoE4 but exhibits essentially no binding to ApoE2 or ApoE3 in the same binding conditions). When one wishes to determine the precise ApoE complement of a patient and whether or not that patient is homozygous or heterozygous for ApoE4, then antibodies which selectively bind to ApoE2 and ApoE3 may also be employed (i.e., an 10 antibody which binds to ApoE2 but exhibits essentially no binding to ApoE3 or ApoE4 in the same binding conditions; an antibody which binds to ApoE3 but exhibits essentially no binding to ApoE2 or ApoE4 in the same binding conditions). Antibodies used to selectively or specifically bind ApoE2, ApoE3, and ApoE4 can be produced by any suitable technique. For example, monoclonal antibodies may be produced in a hybridoma cell line according to 15 the techniques of Kohler and Milstein, *Nature* 265, 495-97 (1975). ApoE2, ApoE3, or ApoE4 may be obtained from a human patient determined to be homozygous therefore, then purified by the technique described in S. Rallet al., *Methods in Enzymol.* 128, 273 (1986), and used as the immunogen for the production of monoclonal or polyclonal antibodies. Purified ApoE isoforms may be produced by recombinant means to express a biologically 20 active isoform, or even an immunogenic fragment thereof may be used as an immunogen. Monoclonal Fab fragments may be produced in *Escherichia coli* from the known sequences by recombinant techniques known to those skilled in the art. See, e.g., W. Huse, *Science* 246, 1275-81 (1989) (recombinant Fab techniques); P. Wenham et al., *Lancet* 337, 1158 (1991) (ApoE PCR primers). The DNA encoding one subtype of ApoE can be obtained and 25 converted to the other by site-directed mutagenesis. See, e.g., T. Kunkel et al., *Methods in Enzymol.* 154, 367-382 (1987); T. Kunkel, U.S. Pat. No. 4,873,192.

The term "antibodies" as used herein refers to all types of immunoglobulins, including IgG, IgM, IgA, IgD, and IgE. The antibodies may be monoclonal or polyclonal and may be of any species of origin, including (for example) mouse, rat, rabbit, horse, or 30 human, or may be chimeric antibodies, and include antibody fragments such as, for example, Fab, F(ab')₂, and Fv fragments, and the corresponding fragments obtained from antibodies other than IgG. For this invention, an antibody selectively or specifically binding ApoE or a particular ApoE isoform (ligand) generally refers to a molecule capable of reacting with or

otherwise recognizing or binding such a ligand. An antibody has binding affinity for a ligand or is specific for a ligand if the antibody binds or is capable of binding the ligand as measured or determined by standard antibody-antigen or ligand-receptor assays, for example, competitive assays, saturation assays, or standard immunoassays such as ELISA or 5 RIA. This definition of specificity applies to single heavy and/or light chains, CDRs, fusion proteins or fragments of heavy and/or light chains, that are specific for the ligand if they bind the ligand alone or in combination.

Antibody assays (immunoassays) may, in general, be homogeneous assays or heterogeneous assays. In a homogeneous assay the immunological reaction usually involves 10 the specific antibody, a labeled analyte, and the sample of interest. The signal arising from the label is modified, directly or indirectly, upon the binding of the antibody to the labeled analyte. Both the immunological reaction and detection of the extent thereof are carried out in a homogeneous solution. Immunochemical labels which may be employed include free radicals, radioisotopes, fluorescent dyes, enzymes, bacteriophages, coenzymes, 15 and so forth.

In a heterogeneous assay approach, the reagents are usually the specimen, the antibody of the invention and a system or means for producing a detectable signal. Similar specimens as described above may be used. The antibody is generally immobilized on a support, such as a bead, plate or Slide, and contacted with the specimen suspected of 20 containing the antigen in a liquid phase. The support is then separated from the liquid phase and either the support phase or the liquid phase is examined for a detectable signal employing means for producing such signal. The signal is related to the presence of the analyte in the specimen. Means for producing a detectable signal include the use of radioactive labels, fluorescent labels, enzyme labels, and so forth. For example, if the antigen 25 to be detected contains a second binding site, an antibody which binds to that site can be conjugated to a detectable group and added to the liquid phase reaction solution before the separation step. The presence of the detectable group on the solid support indicates the presence of the antigen in the test sample. Examples of suitable immunoassays are the radioimmunoassay, immunofluorescence methods, enzyme-linked immunoassays, and the 30 like.

Those skilled in the art will be familiar with numerous specific immunoassay formats and variations thereof which may be useful for carrying out the method disclosed herein. See generally E. Maggio, *Enzyme-Immunoassay*, (1980) (CRC Press, Inc., Boca Raton, Fl.); see

also U.S. Pat. No. 4,727,022 to Skold et al. titled "Methods for Modulating Ligand-Receptor Interactions and their Application," U.S. Pat. No. 4,659,678 to Forrest et al., U.S. Patent No. 4,376,110 to David et al., U.S. Pat. No. 4,275,149 to Litman et al., U.S. Pat. No. 4,233,402 to Maggio et al., and U.S. Pat. No. 4,230,767 to Boguslaski et al. Antibodies which 5 selectively bind an ApoE isoform (i.e., bind to one of ApoE2, ApoE3 or ApoE4 while showing essentially no binding to the other under the same binding conditions) may be conjugated to a solid support suitable for a diagnostic assay (e.g., beads, plates, slides or wells formed from materials such as latex or polystyrene) in accordance with known techniques, such as precipitation. Antibodies which bind an ApoE isoform may likewise be 10 conjugated to detectable groups such as radiolabels (e.g., ³⁵ S, ¹²⁵ I, ¹³¹ I), enzyme labels (e.g., horseradish peroxidase, alkaline phosphatase), and fluorescent labels (e.g., fluorescein) in accordance with known techniques.

In certain embodiments, a kit for detecting for the presence or absence of ApoE4 is employed, where the kit may include one or more reagents for carrying out one or more of 15 the above described ApoE4 isoform, e.g., a nucleic acid for detection of the ApoE4 gene, an ApoE4 specific antibody, etc.

Methods and kits for use in detecting the presence of the ApoE4 isoform are further described in U.S. Patent No. 5,508,167, the disclosure of which is herein incorporated by reference.

20 Where the subject methods include a step of detecting whether the subject carries the ApoE4 allele, in certain embodiments this step typically occurs before administration of the androgenic agent, such that the appropriateness of androgenic therapy is determined prior to administration of the androgen agent. In other words, the subject is first screened for the presence of the ApoE4 allele. If the screen is positive, androgenic therapy is determined to 25 be appropriate. If the screen is negative, androgenic therapy is determined to be not appropriate and is not commenced. In this step, the subject may be determined to be heterozygous or homozygous for the ApoE4 allele.

UTILITY

30 The subject methods find use in any application where the improvement of a cognitive function is desired. The subject methods find use in the treatment of cognitive conditions in a variety of different hosts. As such, the subject methods find use in the treatment of female hosts, as described above, suffering from a cognitive decline disease

condition. The subject methods also find use in the treatment of ApoE4 allele containing hosts, e.g., male or female hosts.

By cognitive decline disease condition is meant a disease condition which is characterized by a deterioration or worsening of at least one cognitive function, such as a 5 deterioration in memory, a deterioration in learning ability, etc. One particular class of cognitive decline diseases which may be treated according to the subject methods are neurodegenerative disease conditions, and more particularly adult onset neurodegenerative disease conditions, such as Alzheimer's disease.

By treatment is meant at least an amelioration of the symptoms associated with the 10 pathological condition afflicting the host, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, e.g. symptom, associated with the pathological condition being treated, such as deterioration in memory or learning ability or other cognitive function. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, 15 e.g. prevented from happening, or stopped, e.g. terminated, such that the host no longer suffers from the pathological condition, or at least the symptoms that characterize the pathological condition.

As mentioned above, in these applications an effective amount of androgenic agent is administered to the host. By "effective amount" is meant a dosage sufficient to produce a 20 desired result, where the desired result is generally an amelioration or alleviation, if not complete cessation, of one or more symptoms of the neurodegenerative disease being treated, particularly the cognitive decline symptoms, e.g., memory decline, learning ability decline, and the like.

The subject methods also find use in the treatment of apoE4-associated disorders. As 25 used herein, an "apoE4-associated disorder" is any disorder that is caused by the presence of apoE4 in a cell, in the serum, in the interstitial fluid, in the cerebrospinal fluid, or in any other bodily fluid of an individual; any physiological process or metabolic event that is influenced by apoE4 domain interaction; any disorder that is characterized by the presence of apoE4; a symptom of a disorder that is caused by the presence of apoE4 in a cell or in a 30 bodily fluid; a phenomenon associated with a disorder caused by the presence in a cell or in a bodily fluid of apoE4; and the sequelae of any disorder that is caused by the presence of apoE4. ApoE4-associated disorders include apoE4-associated neurological disorders and disorders related to high serum lipid levels. ApoE4-associated neurological disorders

include, but are not limited to, sporadic Alzheimer's disease; familial Alzheimer's disease; poor outcome following a stroke; poor outcome following traumatic head injury; and cerebral ischemia. Phenomena associated with apoE4-associated neurological disorders include, but are not limited to, neurofibrillary tangles; amyloid deposits; memory loss; and a 5 reduction in cognitive function, as described in greater detail above. ApoE4-related disorders associated with high serum lipid levels include, but are not limited to, atherosclerosis, and coronary artery disease. Phenomena associated with such apoE4-associated disorders include high serum cholesterol levels.

As used herein, the terms "treatment", "treating", and the like, refer to obtaining a 10 desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse affect attributable to the disease. "Treatment", as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject 15 which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

In addition to the above methods of treatment, the subject methods also find use in 20 the prophylactic or preventative treatment regimens. In such methods, the host is administered an amount of an androgenic agent, typically according to a dosage schedule (e.g., daily, weekly, monthly etc.), that is sufficient to prevent the occurrence of at least 25 symptoms of the ApoE4 associated disorder, e.g., decline in cognition. Thus, the host may first be identified as being at risk for the disorder, e.g., cognitive decline condition, for example by testing for the presence of ApoE4 allele. Following identification of being at 30 risk, the host is commenced on androgenic therapy to prevent or at least slow the occurrence of the disorder or symptoms associated therewith.

KITS

Kits with unit doses of the active androgenic agent, usually in slow-release devices 30 (e.g., either patches or, alternatively, implants under the skin), oral or injectable doses, are provided. In such kits, in addition to the containers containing the unit doses will be an informational package insert describing the use and attendant benefits of the drugs in treating cognitive decline condition of interest and/or improving cognitive function. Preferred

compounds and unit doses are those described herein above. In certain kits, also provided are means for detecting the presence of the ApoE4 isoform in a host.

5 The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

We tested whether administration of androgens might be successful for improving cognition in apoE4 female mice. (As described in Raber et al., Proc Natl Acad Sci U S A 10 1998 Sep 1;95(18):10914-9, where the female ApoE4 mice show age dependent spatial memory impairments that are detected from 6 months of age on ward. See also U.S. Patent No. 6,175,097, the disclosure of which is herein incorporated by reference).

15 Six-month-old female *apoE*^{-/-} mice expressing apoE3 or apoE4 in the brain at comparable levels, as described above, were anesthetized, and Silastic capsules filled with androgens (testosterone or dihydrotestosterone) were implanted subcutaneously; controls received placebo capsules (n = 5-11 mice/genotype and treatment). More specifically, Silastic capsules (ID 1.57 mm; OD 3.18 mm, Dow Corning) were filled with testosterone or dihydrotestosterone (Sigma); placebo capsules were empty. Under methoxyflurane or isoflurane anesthesia, the hair in the back of the neck of mice was cleaned with ethanol, a 20 0.5-cm incision was made, a hormone or placebo capsule was implanted subcutaneously, and the incision was closed with sutures.

25 Testosterone and dihydrotestosterone exert androgenic effects by interacting with androgen receptors [Couse, J. Mol. Med. (1998) 76:497-511. In contrast to testosterone, dihydrotestosterone cannot be converted to 17-β estradiol by aromatase. Thus, comparing the effects of testosterone and dihydrotestosterone allows for a differentiation of androgen and estrogen effects. Eight days after implantation of the capsules, the mice were tested in the water maze. Testosterone improved learning and both testosterone and dihydrotestosterone improved memory retention in female apoE4 mice (Fig. 1). While all three groups learned to locate the hidden platform, there was a significant difference between 30 the learning curves of testosterone- and placebo-treated mice during the hidden platform sessions ($P < 0.05$ by repeated measures ANOVA). In the probe trial (platform removed), both testosterone- and dihydrotestosterone-treated, but not placebo-treated, apoE4 mice spent significantly more time searching in the target quadrant than in any of the other

quadrants (* $P < 0.05$, Tukey-Kramer test). Testosterone did not improve learning or spatial memory retention in female apoE3 mice. After the behavioral testing, plasma hormone levels were determined by radioimmunoassay (RIA) to ensure the effectiveness of the implants.

Plasma hormone levels were measured by RIA (testosterone: ICN, Costa Mesa, CA, and 5 dihydrotestosterone: Diagnostic Systems Laboratories, Webster, TX) per manufacturer's instructions. Plasma testosterone levels in testosterone-treated female mice (5.61 ± 0.74 ng/ml) were similar to those in untreated male mice and in men. These results indicate that brief androgen treatments dramatically improves the spatial learning and memory impairments in adult NSE-apoE4 female mice.

10 To determine whether apoE4 exerts gender-dependent detrimental effects also on nonspatial learning and memory, we assessed 6-month-old male and female apoE4, apoE3, wildtype, and *apoε*^{-/-} mice in a novel object recognition test. During the training session, mice were allowed to explore for 15 min an open field containing two objects. For the retention session (24 hours later), they were placed back into the same open field for 15 min, 15 after one of the familiar objects was replaced with a novel object and the other familiar object with an exact replica. The percentage of time the mice spent exploring the novel versus the familiar object relative to the total amount of time they explored either object in the retention session was used as a measure of object recognition memory. In the training session, all groups of mice spent a comparable amount of time exploring each object. In the 20 retention session, only female apoE4 mice showed significant deficits, whereas male apoE4 mice, and male and female apoE3, wildtype, and *apoε*^{-/-} mice had intact object recognition memory. These results demonstrate that apoE4 induces deficits not only in spatial but also in nonspatial hippocampus- and cortex-dependent learning and memory. The resistance of male apoE4 mice against deficits in object recognition memory indicates that AR-dependent 25 pathways protect against apoE4-induced cognitive impairments. The effects of androgens and AR receptor-dependent pathways on apoE4-induced cognitive deficits in our experimental models explain recent clinical observations in humans. Estrogen failed to preserve cognitive function in women with apoE4 [Yaffe, Neurology (2000) 54:1949-1954]. In addition, testosterone, but not estrogen, levels in serum correlated positively with cognitive 30 performance in older women [Barrett-Connor, JAMA (1999) 279:1289-1293] and testosterone therapy improved cognition in surgically menopausal women [Sherwin, Psychoneuroendocrinology (1988) 13: 345-357].

It is evident from the above results and discussion that improved methods for treating cognitive decline, particularly in female hosts, are provided. The subject methods provide an effective means for improving cognitive function, particularly in females suffering from cognitive decline disorders, e.g., neurodegenerative disease such as Alzheimer's etc. As 5 such, the subject methods represent an important contribution to the art.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. The citation of any 10 publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

Although the foregoing invention has been described in some detail by way of 15 illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method of improving a cognitive function of a female host, said method comprising:
5 administering to said female an effective amount of an androgenic agent to improve said cognitive function.
2. The method according to Claim 1, wherein said androgenic agent is a naturally occurring androgen.
- 10 3. The method according to Claim 2, wherein said androgen is testosterone.
4. The method according to Claim 1, wherein said cognitive function is at least one of learning and memory.
- 15 5. The method according to Claim 1, wherein said female host is a human.
6. The method according to Claim 5, wherein said female human has an ApoE4 phenotype.
- 20 7. The method according to Claim 1, wherein said method further comprises assaying said female host for the presence of at least one ApoE4 allele.
8. A method of treating a female host for a cognitive decline disease condition, said method comprising:
25 administering to said host an effective amount of an androgenic agent to treat said cognitive decline disease condition.
9. The method according to Claim 8, wherein said androgenic agent is a naturally occurring androgen.
- 30 10. The method according to Claim 9, wherein said androgen is testosterone.

11. The method according to Claim 8, wherein said cognitive function is at least one of learning and memory.
12. The method according to Claim 8, wherein said female host is a human.
5
13. The method according to Claim 12, wherein said female human has an ApoE4 phenotype.
14. The method according to Claim 8, wherein said cognitive decline disease condition is
10 a neurodegenerative disease condition.
15. The method according to Claim 14, wherein said neurodegenerative disease condition is Alzheimer's disease.
- 15 16. The method according to Claim 8, wherein said method further comprises assaying said female host for the presence of at least one ApoE4 allele.
17. A method of improving a cognitive function of a host having an ApoE4 allele, said method comprising:
20 administering to said ApoE4 allele containing host an effective amount of an androgenic agent to improve said cognitive function.
18. The method according to Claim 17, wherein said host suffers from a cognitive decline disease condition and said method is a method of treating said host for said cognitive
25 decline disease condition.
19. The method according to Claim 17, wherein said method further comprises assaying said host for the presence of said ApoE4 allele.
- 30 20. A method of treating a host having an ApoE4 associated disorder, said method comprising:
administering to said host an effective amount of an androgenic agent to treat said disorder.

21. The method according to Claim 20, wherein said method further comprises assaying said host for the presence of an ApoE4 allele.
22. A kit for use in treating a female host for a cognitive decline disease condition, said kit comprising:
 - an androgenic agent in a pharmaceutically acceptable vehicle.
23. The kit according to Claim 22, wherein said kit further comprises a means for detecting the presence of at least one ApoE4 allele in said female host.

10

24. The kit according to Claim 22, wherein said androgenic agent is a naturally occurring androgen.
25. The kit according to Claim 22, wherein said kit further comprises instructions for treating said cognitive decline disease condition.
26. A method for preventing the occurrence of a symptom of an ApoE4 associated disorder in a host, said method comprising:
 - administering to said host an effective amount of an androgenic agent to prevent the occurrence of said symptom.
27. The method according to Claim 26, wherein said method further comprising assaying said host for the presence of an ApoE4 allele.

25 28. The method according to Claim 26, wherein said host is a female.

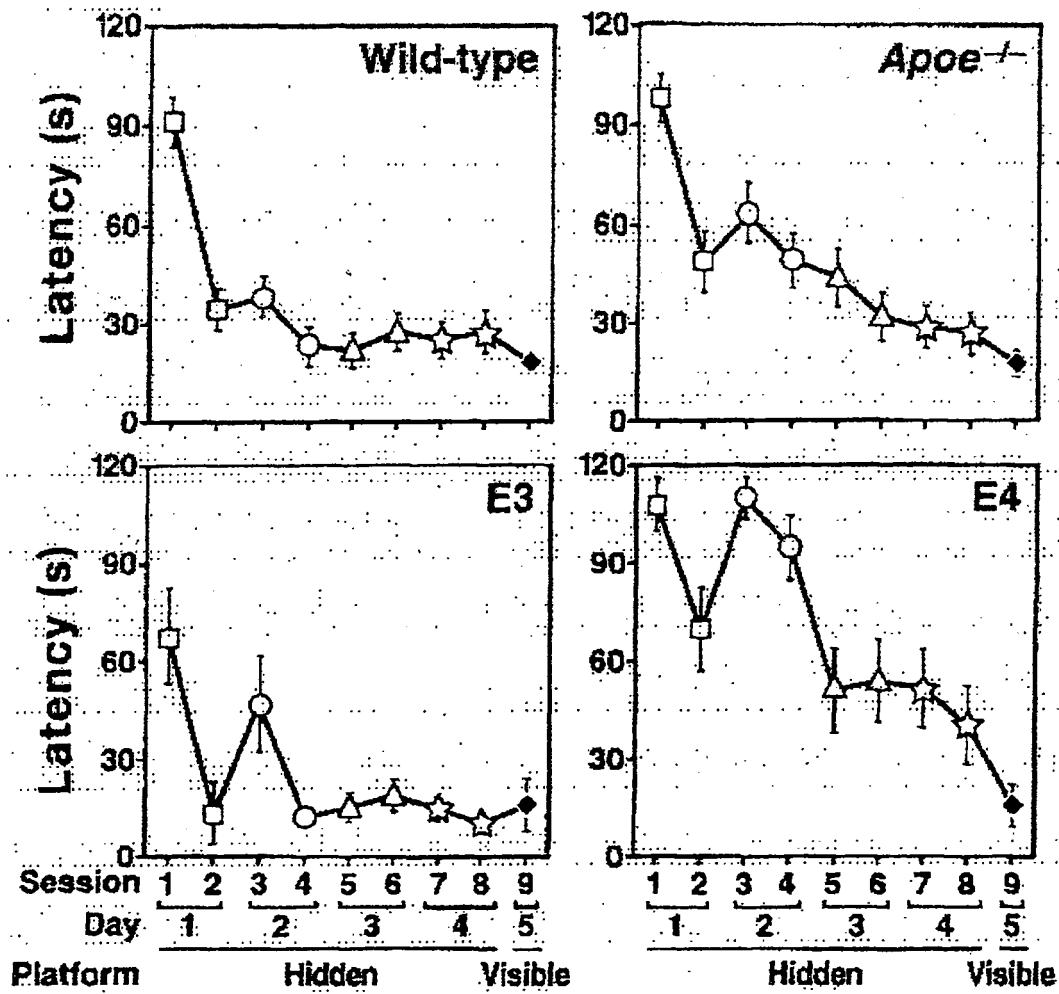


Figure 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/10448

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) A61K 31/56
US CL 514/177, 178

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/177, 178

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: compounds and therapeutic methods, ApoE4

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,791,099 A (AROONSAKUL) 13 December 1988, see columns 3 and 4.	1-5, 8-12, 14-15, 22, 24, 25
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Y		1-28
Y	WO 99 15159 A2 (POIRIER) 01 April 1999, see entire document	1-28

Further documents are listed in the continuation of Box C. See patent family annex.

Special categories of cited documents:	"T"	Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

11 JULY 2001

Date of mailing of the international search report

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